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INCIDENCE OF PRECOCIOUS EGG DEVELOPMENT IN FLIES OF FORENSIC IMPORTANCE (CALLIPHORIDAE)

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Abstract.—A common task of a forensic entomologist is to estimate the postmortem interval (PMI) based on the age of a blow fly larva collected from the corpse. Typically larval age is calculated by comparison to growth curves produced in the laboratory. Previous authors have noted that it is possible for a single blow fly egg to move from one of the ovaries into the vagina and be fertilized before the female has found an oviposition site. Such a developing egg and the resulting larva, which we call "precocious", would appear older compared to its siblings. To our knowledge this phenomenon has not been included in laboratory models of larval growth, raising the possibility that such a larva, if unrecognized, could lead an investigator to overestimate PMI. The relevance of precocious larvae to the practice of forensic entomology depends on how common they are in the field. We dissected and observed the internal reproductive organs of female blow flies caught at decayed meat baits in the San Francisco Bay area. Four species, *Calliphora terraenovae* Macquart, *C. vomitoria* (L.), *C. vicina* Robineau-Desvoidy, and *Lucilia sericata* (Meigen) included individuals found to have a single egg held in the vagina. In the case of *C. terraenovae,* 62% of gravid females contained an egg that had developed to the point where larval spines could be seen through the chorion. Based on these results and previous reports, it appears that precocious eggs are more likely to occur in members of the tribe Calliphorini (the bluebottles) compared to other blow fly lineages. Until this phenomenon is better understood, forensic entomologists may wish to subtract the time required for embryonic development when calculating the minimum possible age of a bluebottle larva.

Key Words.—Insecta forensic entomology, postmortem interval estimation, *Calliphora, Lucilia, Compsomyiops, Phormia.*

Blow fly larvae are the insects most commonly used as evidence in investigations of suspicious death (Smith 1986, Greenberg 1991). The carrion-feeding species very rarely deposit eggs on a live host, therefore the estimated age of a larva is also an estimated minimum postmortem interval (PMI) (Wells and LaMotte 2001). Laboratory growth curves have been developed for a wide variety of species and conditions (e.g., Introna et al. 1989, Goff et al. 1989, Byrd & Butler 1996, Greenberg & Wells 1998), and these usually serve as models of growth in the field.

A female blow fly of those species that have been studied simultaneously matures approximately 200 eggs, and unless the female is disturbed these are all deposited within a period of a few minutes (Hall 1948, Holt et al. 1979, Ives 1988, Wall 1993).

One factor that may complicate the estimation of maggot age when using such data was mentioned by Smith (1986) and discussed in detail by Erzinclioglu (1990). It is possible for a single egg to be held in the vagina (also called the common oviduct), having been fertilized as it passed the spermathecal ducts, in advance of the act of oviposition. That egg can then remain inside the mother, provided that a suitable oviposition site is not immediately found, at least until

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the egg has completed embryonic development. The amount of time that must elapse following egg maturation for this to happen is unknown. The senior author has observed eggs that hatch almost immediately following oviposition in laboratory colonies of Japanese *Aldrichina grahami* (Aldrich) and *Calliphora nigribarbis* Vollenhoven. This occurred when females had been provided a protein meal for egg development during a 24 hour period, and were then given meat for oviposition after three weeks had passed at 20° C. Such extremely "precocious" eggs were not observed among thousands that were obtained approximately 10 days following a protein meal. We suspect that the larva is expelled if it ecloses within the mother, but this also remains to be studied.

It is possible, therefore, for a blow fly to deposit a clutch of eggs in which a single individual immediately hatches while its siblings have just begun embryonic development. As far as we can determine, such precocious eggs have not been included in laboratory studies of maggot growth, and a forensic entomologist using laboratory reference data would overestimate the age of an unrecognized precocious larva by a time period that could be as much as that required for complete embryonic development. Depending on the species and temperature, blow fly embryonic development can require from approximately 14 hours to more than 13 days (Greenberg 1991, Wall et al. 1992, Davies & Ratcliffe 1994).

Erzinclioglu's (1990) report concerned *Calliphora vicina* Robineau-Desvoidy in Britain. Precocious eggs have also been observed in a *C. vicina* laboratory colony from Australia (J. Wallman, personal communication). This species, along with *A. grahami* and *C. nigribarbis,* mentioned above, are all members of the tribe Calliphorini, the bluebottle flies. This raises the possibility that bluebottles are more likely than other blow flies to exhibit this behavior.

Erzinclioglu (1990) seemed to believe that a high proportion of British *C. vicina* carried precocious eggs, although he provided no data. The relevance of precocious blow fly eggs or larvae to the practice of forensic entomology obviously depends upon the probability of encountering one in the field. In this study we recorded the incidence of precocious eggs in blow flies caught at decayed meat baits near the San Francisco Bay.

Materials and Methods

Adult flies were collected at carrion bait using a hand net during the two periods of 14 Sep–4 Nov 1997 and 19 Dec 1997–1 Jan 1998. Collection sites were a number of California locations easily accessible from the city of Berkeley (Alameda CO) including the U.C. Berkeley campus, near China Camp State Park (Marin CO), and near the towns of Bolinas (Marin CO), Fairfax (Marin CO), and Fairfield (Solano CO).

In an effort to prevent the possible expulsion from an adult female of a precocious egg prior to preservation, flies were denied direct access to the bait, which was in a container sealed by tissue paper. Captured flies were also immediately immobilized in 95% ethanol that was kept chilled on dry ice.

Samples were then sorted according to sex and to species using the keys of Hall (1948). Females were dissected and classified as being apparently gravid (eggs appeared to be full size) or not. The presence of an egg in the common oviduct was recorded, and it was also noted if such an egg had completed development to the point where spinous rings were visible (Fig. 1), indicating that embryonic development was nearly complete.

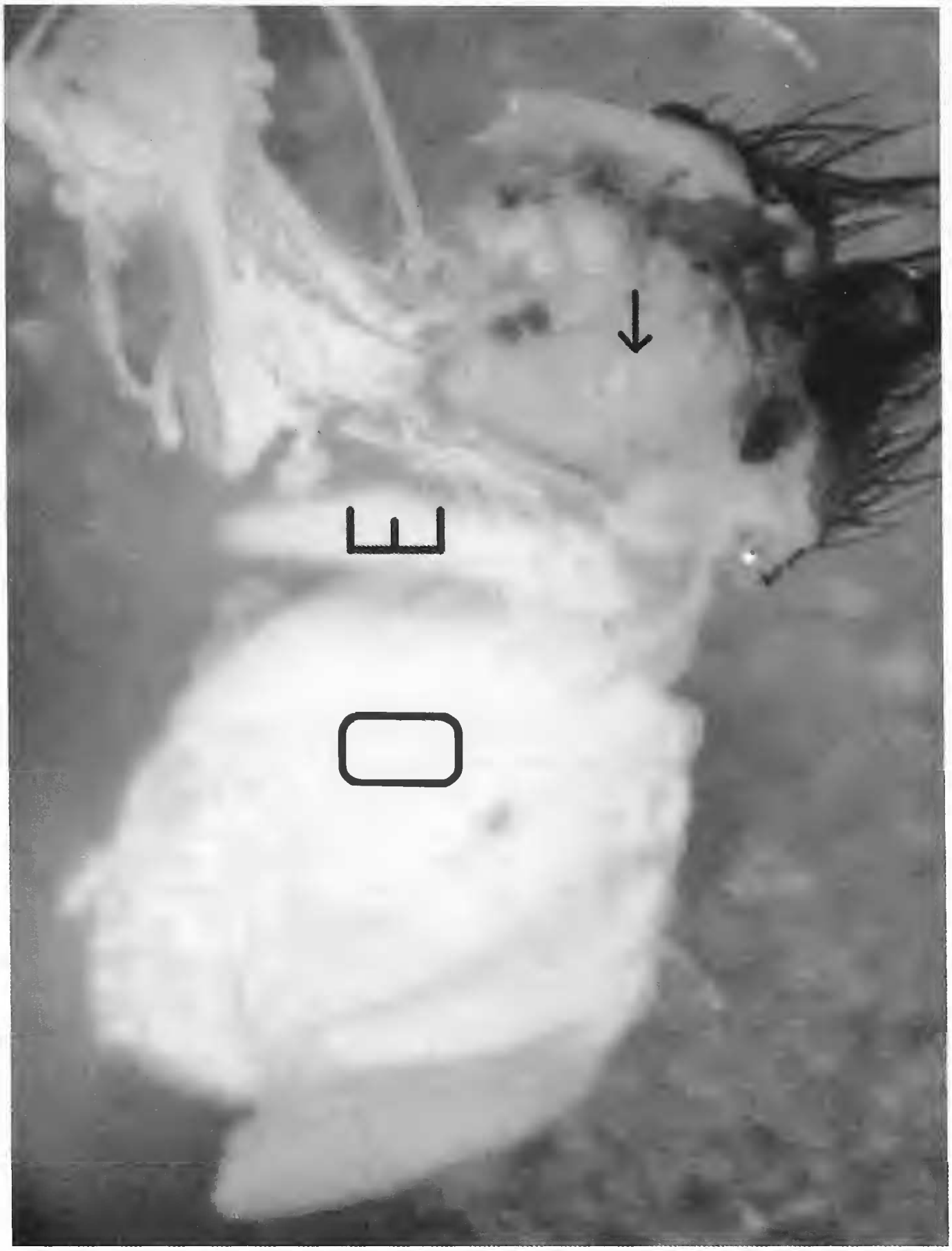

Figure 1. Internal female reproductive system of *Calliphora terraenovae*. The remaining exoskeleton with bristles is from the posterior end of the abdomen. One ovary has been removed. The arrow indicates one spinous ring of developing embryo located in the vagina. O = ovary, E = egg within an individual ovariole.

Results and Discussion

A total of 970 female blow flies were dissected (Table 1). The species observed were *Calliphora vomitoria* (L.), *C. vicina, C. terraenovae* Macquart, *Lucilia* (= *Phaenicia*) *sericata* (Meigen), *Compsomyiops callipes* (Bigot), and *Phormia re-*

Table 1. Egg development in calliphorid flies captured at decayed meat baits near San Francisco Bay. Each column represents a subset of the column to the left.

	Total females	Gravid	Precocious egg[b]	Embryonated[c]
FALL[a]				
C. vomitoria	22	1	0	—
C. vicina	33	27	3	0
L. sericata	197	95	1	0
C. callipes	146	40	0	—
P. regina	319	39	0	—
SUBTOTAL	717			
WINTER[a]				
C. terraenovae	118	55	49	34
C. vomitoria	57	45	3	1
C. vicina	2	2	1	0
L. sericata	3	3	0	—
C. callipes	30	16	0	—
P. regina	43	7	0	—
SUBTOTAL	253			
GRAND TOTAL	970			

[a] Collection periods were of 14 Sep–4 Nov 1997 and 19 Dec 1997–1 Jan 1998.
[b] Located in the vagina rather than in an ovary, and therefore probably fertilized.
[c] Spinous rings of developing larva visible through the chorion.

gina (Meigen). The numbers captured at each location are not shown because we felt that the total number of individuals was too small to draw conclusions about any real differences between study sites. The relatively larger proportion of *Calliphora* spp. in the winter samples is a typical seasonal pattern (Hall 1948).

Precocious eggs were observed in all three *Calliphora* spp. and *L. sericata* (Table 1). In *C. terraenovae* and *C. vomitoria* some eggs had developed to the point that the almost fully formed larva was visible within the chorion. In the case of *C. terraenovae* such obvious embryonic development was seen in 62% of gravid females, i.e., those that would be depositing eggs on a murder victim. Further observations are needed before we can confidently predict the proportion of wild flies carrying an egg that is about to hatch, but it can be quite high.

The single *L. sericata* carrying a precocious egg out of 98 gravid females may represent an anomaly, and we interpret our results as well as earlier observations (see above) to indicate that precocious eggs are much more likely to be found in bluebottles compared to other lineages of carrion-feeding blow flies. We believe that a forensic entomologist must consider these results when using bluebottle larvae to estimate time since death. Erzinclioglu (1990) suggested that when analyzing *C. vicina* present on a corpse the mode rather than the maximum size should be used for PMI estimation. However, Erzinclioglu supported his reasoning with a hypothetical example in which " . . . 500 flies [ovipositing on a corpse produce] 500 larvae that are larger and older than the remaining 150,000 larvae". We interpret this scenario to mean simultaneous oviposition by all females, which seems unlikely. Furthermore non-precocious larvae of equal age can be quite variable in length (Wells & LaMotte 2001) so we wonder how often the distinct size classes described by Erzinclioglu may be found. If fact, we are unaware of

any data showing the distribution of larval sizes corresponding to a particular time since death in a human or other large corpse.

Practicing forensic entomologists may wish to conduct similar surveys of the carrion fly fauna at their location. Until we have a more complete understanding of the incidence of precocious eggs and how they may be recognized in the field, a conservative approach would be to treat any bluebottle larva as potentially precocious. This reasoning would apply no matter how many such larvae are used for the analysis. A minimum age could then be calculated by first using the relevant laboratory larval growth model and then subtracting the time needed for embryonic development.

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